EXHIBIT B

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the use of a poly U primer to synthesize double-stranded molecules with promoters.

Figure 2 shows the use of a 1st strand poly U primer to allow linear isothermal amplification prior to transcription.

Figure 3 shows the use of a 2nd strand ribonucleotide primer to allow linear isothermal amplification prior to transcription.

Figure 4 shows the division of nucleic acid constructs into two groups with non-complementary ends (with SEQ ID).

Figure 5 shows division using three base permutational tails (including SEQ ID).

Figure 6 shows that the oxidation of RNA prevents primer-independent cDNA synthesis by Reverse Transcriptase

Figure 7 shows that the addition of ribonucleotides to the 3' ends of oligonucleotides inhibits subsequent dG-tailing by Terminal Deoxynucleotidyl Transferase

Figure 8 shows the relationship between the efficiency of dG-tailing and the number of rNTPs at the 3' end.

Figure 9 shows the effect of primers with ribonucleotides on RNA yields.

Figure 10 shows the effect of 2' analogues on dG-tailing.

Figure 11 shows the effect of primers with 2' analogues on RNA yields.

there is essentially no further increase in size when dG is attempted to be added after the ribonucleotide modification.

Example 3

Comparison between one and three ribonucleotides for inhibition of dG addition.

Since the product of the TdT mediated addition of U to the primers in the example above probably represents a collection of primers with different numbers of ribonucleotides present instead of a single discrete species, primers were synthesized with either 1 or 3 ribonucleotides already in place at the 3' end. Phosphoramidites for inclusion of ribonucleotide moieties were obtained from Glen Research (Sterling, VA). The sequences of these primers are as follows, where the 5' end comprises a T7 RNA polymerase promoter sequence and the 3' end is complementary to a poly A segment:

PRO - T_{24} = SEQ ID 1

5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG TTT TTT TTT TTT TTT TTT TTT TTT-3'

PRO - $T_{23}U_1$ = SEQ ID 2

5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG TTT TTT TTT TTT TTT TTT TTT TTU-3'

PRO - $T_{21}U_3$ = SEQ ID 3

5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG TTT TTT TTT TTT TTT TTT TTT UUU-3'

Tailing reactions (8µl) were carried out with 80 pmoles of either PRO-T_{24}, PRO-$T_{23}U_1$ or PRO-$T_{21}U_3$ in 1x TdT buffer, 1mM cobalt chloride and 24U TdT for 15' at 37°C, in the presence or absence of 0.05mM dGTP. Aliquots of each reaction

RNA polymerase promoter sequence and the 3' end is complementary to a poly A segment:

PRO - $T_{21}OMe_3$ SEQ ID 4

5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG TTT TTT TTT TTT TTT TTT TTT XXX-3'

where X = 2'-O-Me-U

PRO - $T_{21}Fl_3$ SEQ ID 5

5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG TTT TTT TTT TTT TTT TTT TTT YYY-3'

where Y = 2'-Fl-U

PRO - $T_{22}Fl_2$ SEQ ID 6

5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG TTT TTT TTT TTT TTT TTT TTT TYY-3'

Where Y = 2'-Fl-U

Tailing reactions (8µl) were carried out with 50 pmoles of PRO-T_{24} (from Example 3), PRO - $T_{22}Fl_2$, PRO - $T_{21}Fl_3$ or PRO - $T_{21}OMe_3$ in 8µl reaction mixtures containing 1x TdT Buffer, 1 mM cobalt chloride, 0.05 mM dGTP, 24U TdT, for 15' at 37°C. As controls, reactions were carried out as above with the addition of EDTA to 31mM. Aliquots of each reaction were denatured as described previously and nucleic acids were separated by 10% PAGE containing 7.5 M Urea. Nucleic acids were visualized by staining with ethidium bromide.

Results

The results of this experiment are shown in Figure 7. The reactions with EDTA serve as negative controls to show the positions of the oligonucleotides in the absence of terminal addition. In the positive control with a standard primer, there can be seen a substantial shift in position after dG addition (lane 1 compared to

37°C for 4 hours using the BioArray High-Yield RNA Transcript Labeling Kit (Enzo) with unlabeled nucleotides (3.75mM of each NTP). RNA was purified by the RNeasy Kit as suggested by the manufacturer. One-tenth of each of the eluted RNAs was denatured at 65°C in a formamide loading buffer, electrophoresed on a 1.2% agarose gel (0.5 xTBE), stained with ethidium bromide, visualized with a Kodak Image Station and quantified using Kodak software.

Results

These reactions were carried out in duplicate and the transcription results are depicted in Figure 10 with the quantification shown below the gel picture. Although the effect is not as large as seen in Example 7, the results of this example show an enhancement in production of transcripts of all 4 RNA products even though the reaction never involved a Terminal Deoxynucleotidyl Transferase addition step.

Example 9

Use of homopolymeric ribopolymer as a primer for 1st strand synthesis.

In this example two primers will be used that have the following sequences:

The primer for first strand synthesis U_{24} would be comprised entirely of ribonucleotides:

U_{24} = SEQ ID 7

5' UUU UUU UUU UUU UUU UUU UUU UUU 3'

The primer for second stand synthesis would comprise an RNA promoter sequence and comprise the sequence:

PRO – G_{12} = SEQ ID 8

5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG GGG GGG GGG G-3'